

**A STUDY OF MICROORGANISMS CAUSING DEFECTS IN
DAIRY PRODUCTS, WITH SPECIAL REFERENCE TO
NON-SPORE-FORMING THERMODURIC BACTERIA
FOUND IN PASTEURIZED MILK**

BY

ARTHUR CHARLES MAACK

B. S., Rutgers University, 1936

M. S., University of Illinois, 1938

AN ABSTRACT OF A THESIS

**SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN DAIRY HUSBANDRY
IN THE GRADUATE SCHOOL OF THE UNIVERSITY
OF ILLINOIS, 1943**

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INTRODUCTION

Thermophilic bacteria are frequently a cause of high bacteria counts in pasteurized milk. The term thermophilic, as used here, refers to bacteria which are non-spore-forming organisms capable of surviving to the extent of 10 per cent or more when exposed to 61.7°-62.8°C. for 30 minutes (or 71.1°C. for 15 seconds) in milk. A survival of 10 per cent was used in determining which cultures to include in the study, for if even this percentage of the organisms live through the pasteurization, a milk with a high "raw" count might be thrown into an illegal classification. For example, a count of 1,000,000 per ml. on the raw milk would give a pasteurized count of 100,000 per ml. if there were 10 per cent survival. This would be illegal milk according to most milk ordinances.

These bacteria, as a rule, get into milk on the farm. The milking machine and utensils used in handling milk have frequently been found to be rich sources of contamination. Since chlorine disinfectants are so commonly used for "sterilizing" utensils on the farm, a study was made of the resistance of thermophilic bacteria to these disinfectants. There are a large number of commercial products on the market for dairy use. In general, they are either hypochlorite or chloramine-T products. Since it is a known fact that the presence of alkali affects the germicidal action of chlorine disinfectants, an attempt was made in this study to select both a hypochlorite product containing a large amount of alkali and one with lesser alkali. For the former, *Diversol* was chosen, and for the latter, *B-K* powder was selected. The chloramine-T product was *Santamine*.

The methylene blue reductase test is in common use in the grading of raw milk. For this reason, it is of value to know whether thermophilic bacteria can be detected by this method when they are present in milk in large numbers. Therefore, the reduction of methylene blue by pure cultures of thermophilic bacteria was studied.

Another phase of the study was the classification of the bacteria. In connection with this part of the investigation, observations as to the temperatures at which the cultures would grow were of particular interest because of their practical application to the storage of raw milk.

EXPERIMENTAL PROCEDURE

The cultures used in the study were secured from milk plants which were having high counts in their pasteurized milk, which were believed to be due to thermoduric bacteria. In order to eliminate cultures which were not thermoduric, laboratory pasteurization in milk at 61.7°C. for 30 minutes was carried out. Only those cultures were saved which showed at least 10 per cent survival. Ten of the thermoduric cultures were held at 61.7°C. for a total of four hours in order to determine their resistance to prolonged heating. Plate counts of the milk were made at intervals of 15 minutes.

The studies of the resistance of the cultures to chlorine disinfection were made by washing the growth from an agar slant into sterile, distilled water and then adding 1 ml. of this suspension to 99 ml. of chlorine solution. At the end of $\frac{1}{2}$, 1, 2, 3, 4, and 5 minutes, 0.5 ml. of the solution was withdrawn, added to melted and cooled agar in test tubes, and then plates were poured from these tubes. The strengths of chlorine used for these determinations were 20, 35, 50, 75, and 100 p.p.m. After two days incubation at 32°C. and two days at room temperature observations were made as to whether or not the bacteria had survived.

All of the cultures in this study were first exposed to the action of the highly alkaline hypochlorite at the five concentrations employed. Then, three cultures which were very resistant to the alkaline hypochlorite and three with lesser resistance were exposed to the same concentrations of the "less alkaline" hypochlorite and also to the chloramine-T product.

The reaction (pH) of each strength of the different chlorine solutions was determined on a Coleman electrometer employing a glass electrode.

The study of the ability of the cultures to reduce methylene blue was carried out by inoculating milk, secured directly from the udder of a normal cow with aseptic precautions, with a suspension of the bacteria under observation. Sufficient numbers of bacteria were added so that reduction should have occurred in 4-5 hours. Plate counts were made to check the number of organisms added, and the methylene blue test was run on this milk. Observations were made hourly over a period of 12 hours to determine the time required for reduction.

In the identification of the bacteria the following tests were run: Nitrate reduction, gelatin liquefaction, growth in litmus milk, growth in nutrient broth, indole production, type of growth on agar slants, type of colony formation on agar, type of growth on potato slants, and ability to utilize starch, sucrose, salicin, mannitol, lactose, dextrose, and glycerol as sources of carbohydrates. Morphological studies included motility determinations on the rod

forms, determinations of the shape and arrangement, and staining by the Gram method. The range of temperature supporting growth was also ascertained. Identification was based on Bergey's Manual of Determinative Bacteriology, 5th. Edition.¹

SUMMARY AND CONCLUSIONS

1. Seventy-two cultures of thermophilic bacteria were used in this study. These cultures were obtained from pasteurized milk having high bacteria counts declared to be due to thermophilic bacteria.
2. Ten cultures were subjected to prolonged heating in milk at a temperature of 61.7°C. One culture showed essentially no change in plate count in four hours; all of the cultures showed survival for one hour; five of the cultures showed survival for three hours; and three of the cultures were not destroyed even after four hours of heating. It is concluded that thermophilic bacteria are destroyed very slowly during prolonged heating at 61.7°C.
3. (a) The 72 cultures in the study were exposed to disinfection with a highly alkaline hypochlorite. Eighty-two per cent of the cultures were able to withstand a concentration of 100 p.p.m. available chlorine for 1 minute and 36 per cent a concentration of 50 p.p.m. available chlorine for 2 minutes. Thermophilic bacteria, therefore, are shown to be very resistant to the highly alkaline hypochlorite.

(b) Three cultures were selected which showed considerable resistance to the highly alkaline hypochlorite and three which were much less resistant. These six cultures were then exposed to a "less alkaline" hypochlorite and to a chloramine-T product, using the same procedure as with the highly alkaline hypochlorite. The "less alkaline" hypochlorite was extremely effective, killing all but one of the cultures even at 20 p.p.m. available chlorine for $\frac{1}{2}$ minute exposure. This one culture showed the greatest resistance to the highly alkaline hypochlorite of any of the cultures in the study but only a very slight resistance to the "less alkaline" hypochlorite.

The thermophilic cultures were slightly less resistant to the chloramine-T product than to the highly alkaline hypochlorite. The conclusions to be drawn from this phase of the study are that thermophilic bacteria are not resistant to solutions of hypochlorites of the "less alkaline" type; that they show great resistance to solutions of the highly alkaline hypochlorite; and that they show somewhat less resistance to chloramine-T solutions than to the highly alkaline hypochlorite. Since, when the highly alkaline hypochlorite was used, several of the cultures required an exposure of as much as 50 p.p.m. of available chlorine for 5 minutes in order that complete killing might be effected, it would seem advisable to recommend

that at least this chlorine concentration and time of exposure should be used in sterilizing utensils. A period of 5 minutes may seem too long on the farm or in the milk plant. Therefore, if shorter periods of time are used, greater concentrations of chlorine must be used.

(c) High alkalinity is known to be a factor in reducing the germicidal power of chlorine disinfectants. Therefore, in order to see if there were any relation between reaction (pH) and germicidal power of the chlorine disinfectants in this study, the reaction (pH) of each of the concentrations of the three chlorine disinfectants was determined. It is of interest to note that although the pH of the chloramine-T was 6.65 at a strength of 50 p.p.m. available chlorine, it was considerably less effective than the "less alkaline" hypochlorite, which had a pH of 10.45 at the same chlorine concentration. The pH of the highly alkaline hypochlorite was 11.35 at 50 p.p.m. available chlorine. Therefore, pH did not seem to be the most important factor in determining germicidal power.

4. The methylene blue reductase test was run on pure cultures of fifteen of the thermoduric bacteria. In practically every case reduction required 11-12 hours or longer. Thermoduric bacteria, thus, appear to be poor reducers of methylene blue. In view of this fact, this test can not be used to "grade out" raw milk containing large numbers of these bacteria.
5. The temperature for best growth was determined for the cultures by growing them on agar slants. It was found to be 21°-37°C. About one-fourth of the cultures showed some growth at 10°C. and a similar number at 43°C., but at both temperatures it was not great. It is concluded that the thermoduric bacteria in this study grow best at temperatures of 21°-37°C. and poorly or not at all at 10°C. and 43°C. Therefore, these bacteria probably do not increase appreciably in milk held at 10°C.
6. The identification of the cultures indicated that there were 60 micrococci, 9 rod forms and 1 sarcina. This part of the study was not the phase of major importance. However, a sufficient study of the organisms was made to indicate that the cultures resembled the following species, which are named in the order of their relative abundance: *Micrococcus epidermidis*, 16 cultures; *Micrococcus varians*, 13 cultures; *Micrococcus candidus*, 11 cultures; *Micrococcus flavus*, 8 cultures; *Micrococcus candicans*, 6 cultures; *Microbacterium lacticum*, 5 cultures; *Micrococcus luteus*, 2 cultures; *Flavobacterium lacunatum*, 2 cultures; *Micrococcus subflavus*, 1 culture; *Micrococcus pikowskyi*, 1 culture; *Sarcina lactea*, 1 culture; *Microbacterium mesentericum*, 1 culture; and *Achromobacter healii*, 1 culture. The conclusion is that the predominating species of bacteria in this study were of the Genus *Micrococcus*.

REFERENCE

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VITA

The author was born June 18, 1914, at Dayton, New Jersey. He received his elementary school education in the Dayton Public School and his high school education at New Brunswick (N. J.) High School. From 1932 to 1936 he attended Rutgers University, receiving the Bachelor of Science degree with a major in Dairy Manufactures. He entered the graduate school of the University of Illinois in the Fall of 1936 and received the degree of Master of Science in 1938. He held the position of Assistant in the Dairy Husbandry Department of the University of Illinois from September, 1936, until January, 1941. Since February, 1941, he has held the position of Instructor in the Dairy Husbandry Department at the University of Minnesota. He is a member of Phi Beta Kappa, Gamma Sigma Delta, and Alpha Zeta.

